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Application of dual stable isotopes in investigating food web structure of a naturally disturbed mangrove area of Kelantan Delta, Malaysia

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ABSTRACT

The occurrence of megaflood may significantly alter the natural processes and overall condition in the impacted areas, including estuary locations receiving massive freshwater discharges from terrestrial areas to the sea. The present study is aimed to investigate the food web structure in Kelantan Delta (i.e., a naturally disturbed mangrove area) after the massive disturbance of 2014 megaflood. Various aquatic organisms were collected from the location and all samples were chemically treated according to standard procedures whereby the values of $\delta^{13}C$ and $\delta^{15}N$ ratios were determined by using an elemental analyser - isotope ratio mass spectrometry (EA-IRMS) instrument. The results subsequently showed that the $\delta^{13}C$ ranged between -31‰ and -12‰, whereas the $\delta^{15}N$ ranged between 0.9‰ and 14.8‰. Analyses of $\delta^{13}C$ and $\delta^{15}N$ values revealed four trophic levels whereby the value of $\delta^{15}N$ enrichment to distinguish different trophic levels was 3.5‰. With such amount of trophic levels, areas consistently disturbed would be characterized by short food chains making up their respective food webs. By having such process, Kelantan Delta would have a faster recovery process after any flood event. However, further investigation of the factors promoting population recovery after frequent or massive disturbance should be undertaken to accelerate habitat quality and ecosystem recovery.

Key words : *Kelantan delta, Megaflood, Food web, Stable isotope, Mangrove ecosystem, Food chain length*

Introduction

River flood is an important natural process in the terrestrial area. Accordingly, excessive amount of water containing a significant concentration of dissolved and particulate matters as well as associated contaminants (e.g. trace metals) will be flushed off into the sea. In certain circumstances, a megaflood event can form due to a large fraction of riverine discharges caused by extreme meteorological conditions. This is characterised by short and intense flood events lasting from a few hours to weeks (Velasco *et al.,* 2006). Such event occurred recently in Malaysia whereby an intense discharge from Kelantan River yielded multi-fold amount of suspended materials and brought significant inputs into the mangrove and coastal areas. According to Pour and Hashim (2017), all districts in the State of Kelantan received more than 36,600 mm during this megaflood episode. Regardless, little is known about the immediate effects of megafloods on coastal waters, mainly due to their episodic and unpredictable nature. Meanwhile, some ecological studies have focused on floods as they can cause significant aquatic organism mortality (Fisher *et al.,* 1982).

Food chain length is an important characteristic of ecological communities that may be strongly influenced by any disturbance (Post, 2002a). Its shift can alter ecosystem functions, modify trophic interactions, and affect the biomagnification of contaminants (Carpenter *et al.,* 1987, Cabana and Rasmussen, 1994). The dynamical constraints hypothesis (Pimm, 1982) suggests that longer food chains are less resilient to disturbance, implying that the food chain length will be shorter following a disturbance or in frequently disturbed habitats. Such disturbance is further expected to strongly influence food chain length when it results in the loss and slow return of upper trophic levels (Menge and Sutherland, 1976; Spiller *et al.,* 1998). Prior experiments in small container habitats have shown that long food chains are more susceptible to shortening due to disturbance, and require more time to recolonise after such event (Pimm and Kitching, 1987).

Furthermore, determining the subsequent trophic relationships by using conventional methods typically include approaches such as observing behaviours and undertaking faecal and gut content analyses (Perkin *et al.,* 2014). However, these methods may not reflect the integration of food source items through the food chain; in the cases of a limited timeframe, under or overestimation of source contributions may be observed (Bearhop *et al.,* 2004). Recently, the use of biomarkers from stable isotope analysis has complemented the conventional methods of dietary analysis in examining the trophic relationship and transport of item sources in the food chain (Stowasser *et al.,* 2012; Zulkifli *et al.,* 2014; Chen *et al.,* 2017). A general approach for a stable isotope analysis has thus been suggested to incorporate the ratios of bio-element, namely carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Both stable carbon and nitrogen ratios are potential continuous numerical variables in describing a food web (Zulkifli *et al.,* 2016). In particular, the stable carbon ratio (ratio of $^{13}C/^{12}C$) indicates the feeding and carbon pathway from prey to the predator in a very small value of about 1.0‰ (Zanden and Rasmussen, 2001). Moreover, the consistent enrichment of nitrogen ratio (term of $^{15}N/^{14}N$) shows an increment of 3.4‰ per trophic level, enabling the measurement of trophic fractionation for each organism in a food web (Minagawa and Wada, 1984). Therefore, the present study aims to develop a food web model and determine the length of the Kelantan Delta trophic levels after the massive disturbance of 2014 megaflood via the stable isotope approach.

Materials and Methods

Bimonthly samplings were conducted in the Kelantan Delta area (Figure 1). Zulkifli *et al.* (2010) reported this area has a small size of developed area and low intensity of recreational-, agricultural- and

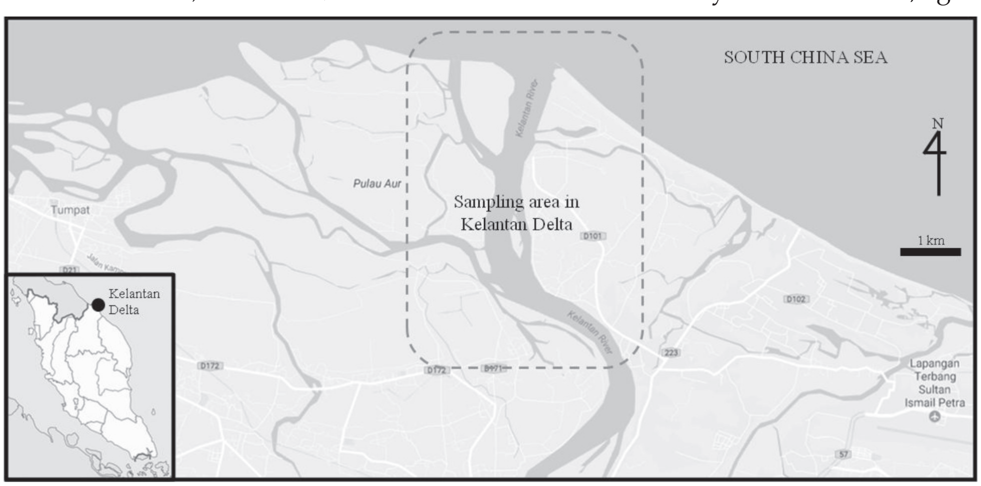

Fig. 1. Sampling area in Kelantan Delta, Malaysia

industrial- activities. However, it is surrounded by many villages, and several jetties used by many fishing vessles. Accordingly, sediment, water, and various types of biological samples were collected from a predetermined location within the delta area. All samples were then transported to the laboratory and kept frozen at -20^{o}C for further analysis. In the laboratory, the frozen samples were thawed to room temperature and subjected to pre-treatment based on the method described by Zulkifli *et al.* (2012). In brief, the tissue samples were washed with Mili-Q water and dried in a freeze dryer for at least 24 h or until a constant weight was obtained. Each dried sample was ground to yield fine powder and added 3 ml chloroform and methanol mixture (2:1 ratio), then for 3 h to eliminate the lipid component. Next, the mixture was centrifuged for 10 min using a high-speed centrifuge (760 x G, 4 ^{o}C) before the supernatant was discarded and the remaining pellet was fumed with 12M hydrochloric acid (HCl) for 10 h to remove inorganic carbonates. Following this, any excess acid contained in the pellet was removed in a vacuum desiccator by exposing to sodium hydroxide (NaOH) pellets for 3 h. The stable carbon and nitrogen ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were determined by using a continuous flow isotope-ratio mass spectrometer with an elemental analyser. Accordingly, the carbon and nitrogen isotope ratios are expressed in delta notation ($\delta^{13}C$ and $\delta^{15}N$) in the unit of parts per thousand (‰):

Delta X (ppt) = [(R of sample/R of standard) – 1] x 1000

where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of sample or standard and X is $\delta^{13}C$ or $\delta^{15}N$ in part per thousand (‰) deviation of the sample from the recognised isotope standard. The analytical precision for the isotopic analyses was better than ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Pee Dee belemnite (PDB) limestone carbonate and atmospheric nitrogen (N_2) were utilised as the standard for carbon and nitrogen isotope ratios, respectively.

The $\delta^{15}N$ values obtained were used to indicate the trophic position for each organism. The following formula is used to calculate the trophic level:

$$TL_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer})/3.4]+2$$

where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ for the consumer species, $\delta^{15}N_{primary\ consumer}$ is the $\delta^{15}N$ reference baseline value at trophic level 2 within the given food web, and 3.4 is the overall average fractionation value per trophic level increase (Minagawa and Wada, 1984). The trophic levels were calculated based on $\delta^{15}N_{primary\ consumer}$ as the baseline. In this case, the mean $\delta^{15}N$ value for trumpet snail (*Faunus* sp.) was taken as the reference as it was the primary consumer.

Results

A total of 33 biological species were analysed and the stable isotope $\delta^{13}C$ and $\delta^{15}N$ ratios present in their tissues were determined accordingly. In general, the $\delta^{13}C$ ranged between -31‰ and -12‰, whereas the $\delta^{15}N$ ranged between 0.9‰ and 14.8‰. Complete data on $\delta^{13}C$ and $\delta^{15}N$ ratios of all samples collected from Kelantan Delta are listed in Table 1. To calculate the trophic level of consumer ($TL_{consumer}$), $\delta^{15}N$ ratio of trumpet snail (*Faunus* sp.) was utilised. The combination of both $\delta^{13}C$ and $\delta^{15}N$ data is demonstrated in Figure 2 accordingly. Among all biological samples, the tiger tooth

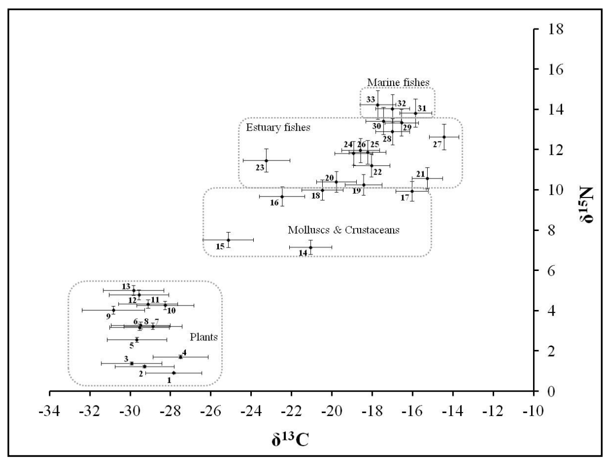

Fig. 2. Distribution of $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios (mean ± standard deviation) of biological samples collected from Kelantan Delta. [Remark: (1) *Nypa fruticans*, (2) *Mimosa pudica*, (3) *Peltophorum pterocarpum*, (4) *Hibiscus tiliaceus*, (5) *Soneratia caseolaris* (sapling), (6) *Cocos nucifera*, (7) *Acrostichum speciosum*, (8) *Ipomoea pes-caprae*, (9) *Soneratia caseolaris*, (10) *Acrostichum aureum*, (11) *Arundo donax*, (12) *Cassytha filiformis*, (13) *Acanthus ilicifolius*, (14) *Faunus* sp., (15) *Mactra grandis*, (16) *Pythia* sp., (17) *Portunus pelagicus* (small), (18) *Macrobrachium rosenbergii*, (19) *Lutjanus johnii*, (20) *Valamugil cunnesius*, (21) *Portunus pelagicus* (big), (22) *Atule mate*, (23) *Litopenaeus setiferus*, (24) *Scatophagus argus*, (25) *Selar boops*, (26) *Gerres filamentosus*, (27) *Epinephelus sexfasciatus* (small),

Table 1. Values of $\delta^{13}C$ and $\delta^{15}N$ (mean±sd), calculated trophic level of consumer and general trophic level of biological samples collected from Kelantan Delta after 2014 megaflood.

Label	Common Name	Scientific Name	$\delta^{13}C$	$\delta^{15}N$	$TL_{consumer}$	TL
1	Nipa Palm	*Nypa fruticans*	-27.84±0.11	0.91±0.04	0.2	1
2	Sleepy Plant	*Mimosa pudica*	-29.30±0.19	1.23±0.95	0.3	1
3	Yellow Flametree	*Peltophorum pterocarpum*	-29.94±0.10	1.39±0.64	0.3	1
4	Sea Hibiscus	*Hibiscus tiliaceus*	-27.51±0.22	1.71±0.81	0.4	1
5	Mangrove Apple (Sapling)	*Soneratia caseolaris*	-29.67±0.33	2.56±0.30	0.7	1
6	Coconut	*Cocos nucifera*	-29.54±0.14	3.18±0.85	0.8	1
7	Mangrove Fern	*Acrostichum speciosum*	-28.88±0.25	3.23±1.26	0.8	1
8	Beach Morning Glory	*Ipomoea pes-caprae*	-29.49±0.20	3.28±0.13	0.9	1
9	Mangrove Apple	*Soneratia caseolaris*	-30.84±0.15	4.03±1.05	1.1	1
10	Golden Leather Fern	*Acrostichum aureum*	-28.28±0.06	4.27±0.82	1.2	1
11	Giant Cane	*Arundo donax*	-29.12±0.12	4.34±1.31	1.2	1
12	Love-Vine	*Cassytha filiformis*	-29.57±0.15	4.80±0.41	1.3	1
13	Holly-Leaved Acanthus	*Acanthus ilicifolius*	-29.84±0.22	5.01±0.60	1.4	1
14	Trumpet Snail	*Faunus* sp.	-21.06±0.20	7.15±0.13	2.0	2
15	Duck Clam	*Mactra grandis*	-25.15±0.16	7.52±0.21	2.1	2
16	Pythia Snail	*Pythia* sp.	-22.47±0.05	9.68±0.03	2.7	2
17	Flower Crab (Small)	*Portunus pelagicus*	-16.03±0.08	9.94±0.29	2.8	2
18	Giant River Prawn	*Macrobrachium rosenbergii*	-20.47±0.37	10.00±0.32	2.8	2
19	John's Snapper	*Lutjanus johnii*	-18.44±0.16	10.26±0.15	3.0	3
20	Longarm Mullet	*Valamugil cunnesius*	-19.78±0.48	10.40±0.24	3.0	3
21	Flower Crab (Big)	*Portunus pelagicus*	-15.28±0.24	10.58±0.10	3.0	3
22	Yellowtail Scad	*Atule mate*	-18.04±0.24	11.22±0.14	3.2	3
23	White Shrimp	*Litopenaeus setiferus*	-23.26±0.26	11.47±0.22	3.3	3
24	Spotted Scat	*Scatophagus argus*	-18.93±0.18	11.82±0.17	3.4	3
25	Oxeye Scad	*Selar boops*	-18.24±0.24	11.87±0.21	3.4	3
26	Whipfin Silver-Biddy	*Gerres filamentosus*	-18.59±0.16	11.97±0.25	3.4	3
27	Sixbar Grouper (Small)	*Epinephelus sexfasciatus*	-14.45±0.04	12.64±0.10	3.6	3
28	Common Ponyfish	*Leiognathus equulus*	-17.00±0.26	12.9±0.15	3.7	3
29	Giant Seacatfish	*Arius thalassinus*	-16.56±0.12	13.34±0.08	3.8	3
30	Javelin Grunter	*Pomadasys kaakan*	-17.45±0.12	13.42±0.06	3.8	3
31	Indian Threadfish	*Alectis indicus*	-15.86±0.12	13.82±0.09	4.0	4
32	Sixbar Grouper (Big)	*Epinephelus sexfasciatus*	-17.00±0.16	14.04±0.07	4.0	4
33	Tigertooth Croaker	*Otolitus ruber*	-17.74±0.01	14.24±0.04	4.1	4

(28) *Leiognathus equulus*, (29) *Arius thalassinus*, (30) *Pomadasys kaakan*, (31) *Alectis indicus*, (32) *Epinephelus sexfasciatus* (big), (33) *Otolitus ruber*]

croaker (*Otolitus ruber*) was positioned at the top (i.e., mean $\delta^{15}N$ = 14.24‰). Meanwhile, global studies on $\delta^{15}N$ showed an enrichment between 2.8‰ and 4.0‰ (i.e., approximate mean value of 3.4‰), which would distinguish the trophic level of one species (Minagawa and Wada, 1984). The food web in Kelantan Delta can be divided into four trophic levels (Figure 3). In particular, the riverside and aquatic plant species formed the group for trophic level 1 (TL1) whereby the $\delta^{13}C$ and $\delta^{15}N$ ratio data ranged approximately from -31‰ to -27‰ and 0.9‰ to 6‰, respectively. Meanwhile, terrestrial plants contain a lower amount of $\delta^{15}N$ ratio if the primary consumer used is an aquatic mollusc. Therefore, the primary consumers made up the trophic level 2 (TL2), which mainly consisted of molluscs and crustaceans, and their $\delta^{13}C$ and $\delta^{15}N$ ratio data ranged approximately from -26‰ to -16‰ and 7‰ to 11‰, respectively. In contrast, the secondary consumers primarily consisted of 12 fish species, thereby forming the trophic level 3 (TL3). Their $\delta^{13}C$ and $\delta^{15}N$ ratio data ranged from -24‰ to -14‰ and 10‰ to 13.4‰, respectively. Finally, the topmost in the food web of Kelantan Delta consisted of three marine fish species, thus forming the trophic level 4 (TL4) and yielded $\delta^{13}C$ and $\delta^{15}N$ ratio data ranged approximately from -18‰ to -15‰ and more than 14‰, respectively.

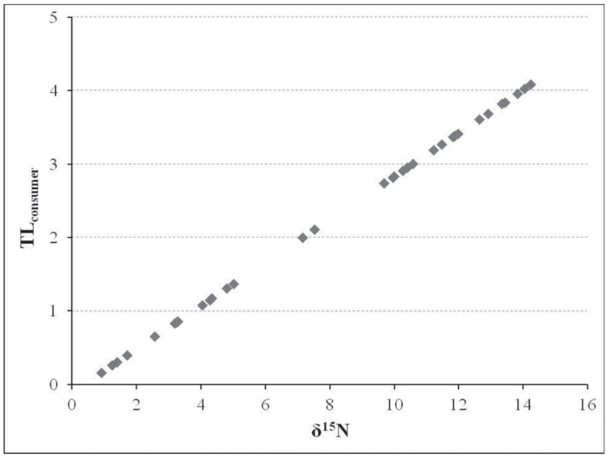

Fig. 3. Relationship between calculated trophic level for each organism with $\delta^{15}N$ values

Discussion

Based on the samples obtained, the $\delta^{15}N$ was enriched at approximately 3.5‰ and thus separated these species into four distinct trophic levels. This value is within the previously reported $\delta^{15}N$ enrichment, which are between 2.8‰ and 4.0‰ (mean value of 3.4‰) as reported by Minagawa and Wada (1984). When compare with that of at the seagrass bed of Sungai Pulai estuary (a Ramsar site located in Johor, Malaysia) as detailed by Mukhtar *et al.* (2016), the trophic levels at Kelantan Delta were fewer. However, the food web length was identical to other disturbed areas, such as the Macao wetlands (Chen *et al.*, 2017), the estuary of Perak River (Roslia *et al.*, 2017) and and at the intertidal mudflat of Johor Strait (Zulkifli *et al.*, 2016). In particular, the organisms at Sungai Pulai estuary have formed a complex food web containing five trophic levels whereby the lesser amount of trophic level indirectly demonstrates the shorter food chains present within it. This is in agreement with Pimm (1982), who has suggested that food chain length will be shorter in the case of a massive disturbance or frequently disturbed habitats. Although it has been hypothesized that a mangrove ecosystem usually is a complex ecosystem and high productivity area (Khan *et al.*, 2017), this condition depends on frequency and degree of disturbance occurring from time to time (Harris *et al.*, 2010). Moreover, Doi and Hillebrand (2019) have speculated that such shorter food chain length can occur at the highest productivity level; this is probably due to the predator switching to feed at the lower trophic levels as a result of the abundant basal resources offered. In terms of the ecosystem, a shorter food chain is capable of a faster recovery following a disturbance (Jenkins *et al.*, 1992). Regardless, further investigation of the factors promoting population recovery after frequent or massive disturbances should be undertaken to accelerate habitat quality and ecosystem recovery.

Conclusion

The 2014 megaflood could potentially cause a massive disturbance on the food web of the locality. The presence of shorter food chains creating the food web in Kelantan Delta allows the ecosystem to naturally and eventually recover. In particular, the $\delta^{13}C$ values obtained ranged between -31‰ and -12‰, while the $\delta^{15}N$ values ranged between 0.9‰ and 14.8 ‰. Additionally, the food chain length in Kelantan Delta reached up to trophic level 4 after the 2014 megaflood disturbance.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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